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INVITED COMMENTARY

Sperm Biology

The use of spermatogonial stem cells to correct a mutation causing meiotic arrest

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The basis of life-long spermatogenesis is spermatogonial stem cells (SSCs) located at the basal membrane of the seminiferous tubules in the testis. After several rounds of proliferation and spermatogonial differentiation, the male germ cells will eventually undergo meiosis to form haploid spermatids. Disturbance of the molecular regulation of spermatogenesis can lead to spermatogenic arrest, in humans often during meiosis,[1,2] and subsequent azoospermia. Unfortunately, no treatment option enabling conception of a genetically own child is currently available for men suffering from spermatogenic arrest before spermatids are formed.

In this issue of the *Asian Journal of Andrology*, Wang *et al.*[3] present the use of cultured SSCs to restore a mutation, which otherwise leads to meiotic arrest, by clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated endonuclease 9 (CRISPR-Cas9)-mediated genome editing. This would theoretically open the possibility to use a patient's own SSCs to correct the mutation that causes meiotic arrest, followed by an autotransplantation to the patient's testes where the corrected SSCs would be able to initiate complete spermatogenesis.[4]

Besides mutations causing meiotic arrest, potentially any mutation that can cause a severe monogenetic disease in the offspring can be corrected in cultured SSCs. Indeed, in 2015, Wu *et al.*[5] were able to correct a mutation in the gene crystallin gamma C (*Crygc*; which leads to blindness when uncorrected) in cultured SSCs. Upon transplantation to testes that lack endogenous spermatogenesis, these restored SSCs were able to find the stem cell niche and generate wild-type spermatids. These spermatids were used to generate offspring without the blindness-causing mutation.[5]

One problem that remains is that, in most clinical settings, the recipient testes still contain endogenous germ cells. These endogenous germ cells still harbor the mutation. Therefore, even after autotransplantation of corrected SSCs, offspring may still be fathered by sperm derived from uncorrected endogenous SSCs. These endogenous SSCs should thus be removed from the patient's testes before autotransplantation of the corrected SSCs. Because this would require irradiation, or genotoxic chemicals such as busulfan, clinical application of autotransplantation of corrected SSCs is currently not feasible.[4]

One exception may be correction of mutations that cause meiotic arrest. In these cases, no endogenous spermatids are formed, so all offspring will be derived from germ cells with the corrected gene. Wang *et al.*[3] have now generated mice that harbor a mutation in the gene testis expressed 11 (*Tex11*) that mimics a similar mutation that causes meiotic arrest in humans. As published for the *Crygc* gene,[5] CRISPR-Cas9 was applied to correct the *Tex11* mutation followed by transplantation to recipient testes lacking endogenous spermatogenesis. As expected, this enabled wild-type spermatogenesis in the recipient testes and generation of fertile offspring.

However, typically, a human male harboring a mutation in the *TEX11* gene, or any patient suffering from meiotic arrest, still has his endogenous germ cells up until the spermatocyte stage before the arrest. Although not generating sperm, these endogenous germ cells may still hinder homing of the autotransplanted SSCs to the basal membrane of the seminiferous tubules. The question thus is whether autotransplanted corrected SSCs will reach the basal membrane of the seminiferous tubules, from where they can start to grow and differentiate, when the patient's endogenous germ cells potentially block the way. To answer this question, Wang *et al.*[3] also transplanted corrected spermatogonia to *Tex11*-mutated testes that contain germ cells up until the arrested spermatocyte stage. Importantly, in this case, they did not find any corrected SSCs to reach the basal membrane and no complete spermatogenesis was observed after the SSC transplantation. Hence, the endogenous germ cells hindered homing, and thus subsequent spermatogenesis, of the corrected SSCs.

For further development of this method for possible clinical application, this is a crucial finding. Removal of the endogenous germ cells from the patient's testes by irradiation or genotoxic chemicals does not seem to be a healthy solution at this moment. Perhaps it is not the germ cells themselves but an intact blood–testis barrier that hinders homing of the corrected SSCs. The blood–testis barrier consists of Sertoli–germ cell junctions, as well as Sertoli–Sertoli cell junctions that divide the seminiferous epithelium into a basal and adluminal compartment.[6] Perhaps, development of a way to temporally disturb this barrier would enable homing of the corrected SSCs. Another option may be use of the genetically corrected SSCs to generate sperm *in vitro*, thereby omitting the need for transplantation.[7,8] However, the methods to achieve this have not been sufficiently developed. For instance, the meiotic checkpoints that are normally active during meiosis *in vivo* to prevent genomic aberrations to be transmitted to the offspring are not necessarily also active *in vitro*.[8]

Hence, Wang *et al.*[3] have perfectly illustrated the possibilities and problems of using autotransplantation of genetically corrected male germline stem cells to "restore" spermatogenesis in men suffering from meiotic arrest. To our knowledge, their paper is the first to demonstrate that endogenous germ cells can block homing of transplanted SSCs. Although this may feel as a setback, this piece of information is crucial for further consideration and development of a treatment option for male meiotic arrest patients that is based on autotransplantation of SSCs.

COMPETING INTERESTS

Both authors declare no competing interests.

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